

Rapid Screening and Confirmation of Melamine Residues in Milk and Its Products by Liquid Chromatography Tandem Mass Spectrometry

Application Note

Food

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Abstract

This rapid method uses the Agilent 6410 Triple Quadrupole (QQQ) with a cation ion exchange column for the liquid chromatography tandem mass spectrometry (LC/MS/MS) analysis of dairy products for melamine. Milk and milk products are prepared with a simple SPE cleanup method employing the new Agilent SampliQ SCX cartridge. The residue is quantified in the multiple reaction monitoring (MRM) mode. The selectivity of the QQQ can easily eliminate any matrix interferences that may occur in the separation and provide excellent response. The method provides good results with respect to precision, repeatability, and spiked recovery. The recovery of 80 ppb and 50 ppb melamine spikes in milk powder using the external standard calculation is 62.5 and 83.4 percent, respectively, and the RSD is less than 3 percent.



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Introduction

Melamine, a nitrogen-based compound used in industrial and commercial plastics, can cause kidney failure, and has been found in infant formula and other milk products.

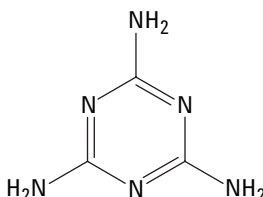


Figure.1 Structure of melamine $C_3H_6N_6$, $MW = 126.1199$.

In this work, a highly selective, sensitive LC/MS/MS method is developed and, compared to the GC/MS method, requires no derivatization. The method can both confirm and quantify in a single analysis and can achieve very low detection limits in complex matrices such as milk and milk products.

The ZORBAX 300SCX ion-exchange column is simple, fast, and equivalent to the column used in the China GB method and can easily meet the analysis requirements. Therefore, the method can improve lab efficiency/productivity and obtain more reliable and defendable results. It is suitable for the confirmation and quantitation for positive result screening by HPLC.

Experimental

Reagents and Chemicals

The acetonitrile is HPLC grade purchase from Dikma (Beijing, China). The HPLC water is prepared with a Milli-Q system.

Trichloroacetic acid solution: Weigh 50 g trichloroacetic acid and dissolve into 1 L of water.

Ammoniacal methanol solution: Weigh 5 mL ammonium hydroxide and 95 mL methanol

LC Parameters

Column	Agilent ZORBAX 300SCX, 2.1 mm \times 150 mm, 5 μ m (p/n 883700-704)
Injection volume	10 μ L
Flow rate	0.2 mL/min
Temperature	40 $^{\circ}$ C
Mobile phase	A: 10 mM NH_4 acetate/acetic acid pH adjusted to 3.0 B: ACN A:B = 20:80
Stop time	10 min

MS Parameters

Agilent 6410A LC/MS Triple Quadrupole	
Ion source	Electrospray
Polarity	Positive
Nebulizer gas	Nitrogen
Ion spray voltage	4000 V
Dry gas temperature	350 $^{\circ}$ C
Dry gas flow rate	9 L/min
Nebulizer pressure	40 psi
Resolution	Q1 (unit) Q3 (unit)

MRM Setting

Rt	Compound	Precursor	Product	Dwell (ms)	Fragmentor (V)	Collision Energy (V)
7 min	Melamine	127	85	200	100	20
		127	68	200	100	35

Sample Preparation

- Standards solution: dissolve melamine into mobile phase to concentration level at 1, 5, 10, 50, 100, and 500 ppb.
- Liquid milk, milk powder, yogurt and ice-cream sample preparation:

2.1 Extraction

Weigh 2 g of the sample into a 50-mL plastic centrifuge tube with cup, add 15 mL 5% trichloroacetic acid in water solution and 5 mL acetonitrile, sonicate for 10 min, vortex for 10 min, and then centrifuge 10 min at 4000 rpm. Wet filter paper with 5% trichloroacetic acid and filter the supernatant and using a 25.0-mL volumetric flask, bring to volume with 5% trichloroacetic acid solution. Transfer 5.0 mL and then add 5 mL water for further cleanup.

2.2 SPE Cleanup

Load the above solution onto the SPE cartridge, 6 mL/150 mg SampliQ SCX (p/n 5982-3267). Condition the SPE cartridge before use by washing with 5 mL methanol and then 6 mL water to activate. After loading the sample wash with 5 mL water and then 5 mL methanol, vacuum to almost dry, and elute with 5 mL 5% ammoniacal methanol solution. Control the flow rate at less than 1 mL/min. Dry the eluate under 50 $^{\circ}$ C nitrogen. Then dissolve the residue (equivalent 0.4 g sample) with 1.0 mL mobile phase, vortex 1 min, and filter via 0.2 μ regenerated cellulose membrane filter (p/n 5064-8222) before injection.

Results and Discussion

Milk and relevant milk products contain hundreds of compounds and it is necessary to distinguish the illicitly added melamine among them. For confirmation and accurate quantification beyond the capability of LC, the LC/MS/MS method is an excellent tool. The high selectivity of the first and third quadrupoles, each working as a “mass filter” in MRM mode, allows selection of the precursor ion and two characteristic fragments, one used for quantitation and the other a qualifier ion (with both present at the corresponding ratio to the standard providing confirmation). Thus, mass spectrometry is a

tool that can provide melamine screening laboratories and dairy product manufacturers accurate and precise quantitation and confirmation for samples screened positive.

The chromatograms in Figure 3 show that the Agilent 6410 QQQ determination removes chemical interferences using the high selectivity of the LC/MS/MS in MRM mode. Both the quantitation ion and the qualifier ion have little noise and no matrix interfering peaks, providing accurate and precise quantitation and confirmation. Confirmation is obtained by comparing both the ion ratios and the retention time to the results obtained for melamine standards.

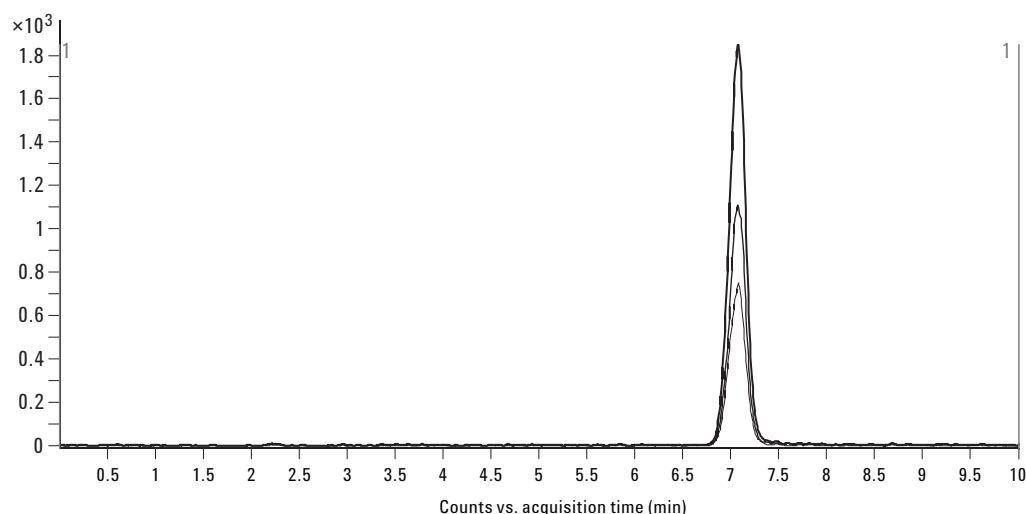


Figure 2. Result in solvent showing the precursor ion and two transition ions at the 10 ppb level.

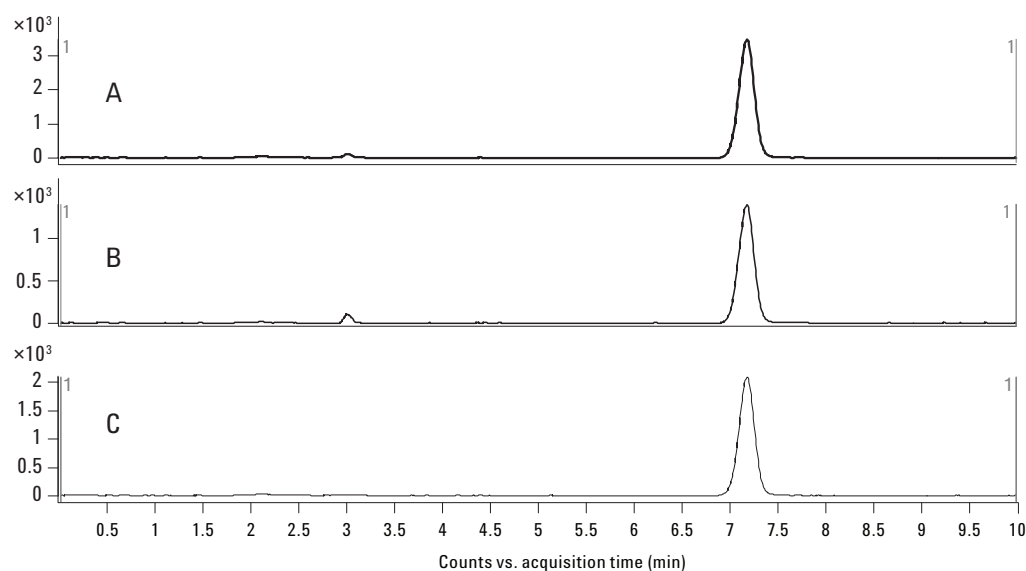


Figure 3. Results of 18.3 ppb level in milk powder with a 10 μ L injection, A) total ion chromatogram (TIC), B) qualifier ion, and C) quantitation ion.

Linearity

Another advantage of QQQ technology is the wide dynamic range for the different levels of sample concentrations as seen in Figure 4.

As shown in Figure 3, it is quite easy to obtain very low detection and, at the same time, analyze high-concentration samples. Samples screened positive by LC/UV detection with possible melamine concentrations above ppm level should be diluted for LC/MS/MS confirmation to avoid contamination of the highly sensitive MS system.

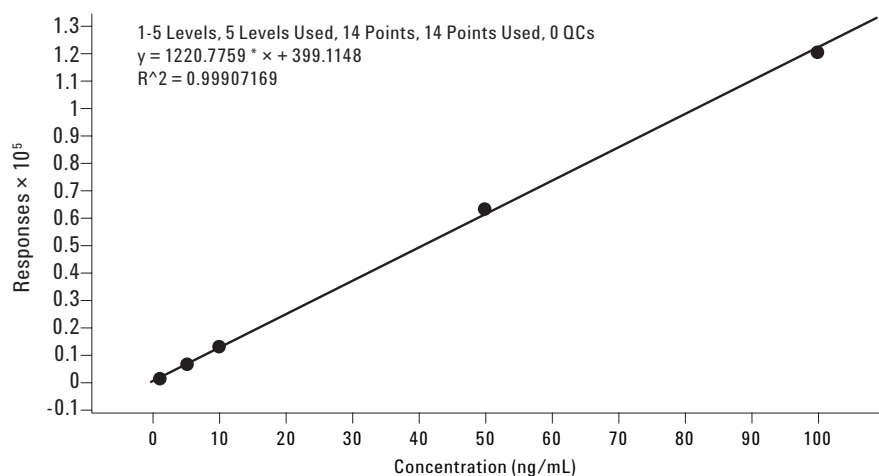


Figure 4. Linearity result for melamine using the MRM quantitation ion.

Sensitivity

Using LC/MS/MS excellent sensitivity can be obtained even in complex and dirty matrices. There is almost no background even at very low levels in milk samples.

Figure 5. shows a milk sample spiked at 1 ppb with melamine. Using these data, the calculated result for the limit of quantitation (LOQ) (S/N >10 peak to peak) is 0.5 ppb and limit of detection (LOD) (S/N > 3 peak to peak) is 0.2 ppb.

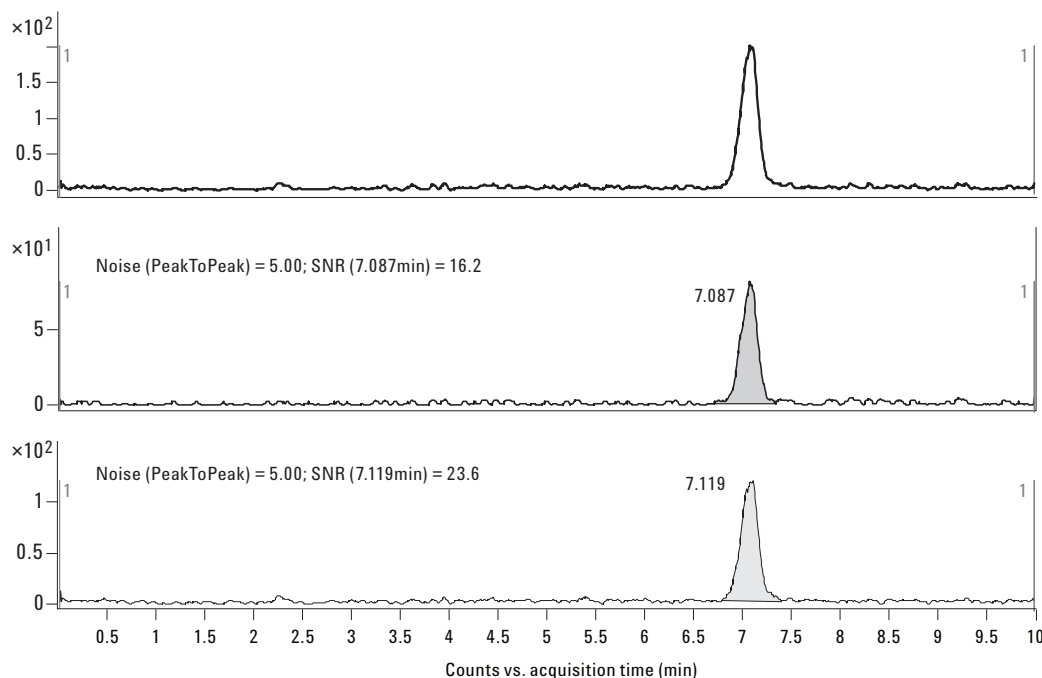


Figure 5. Response of melamine in a milk sample spiked at 1 ppb.

Repeatability

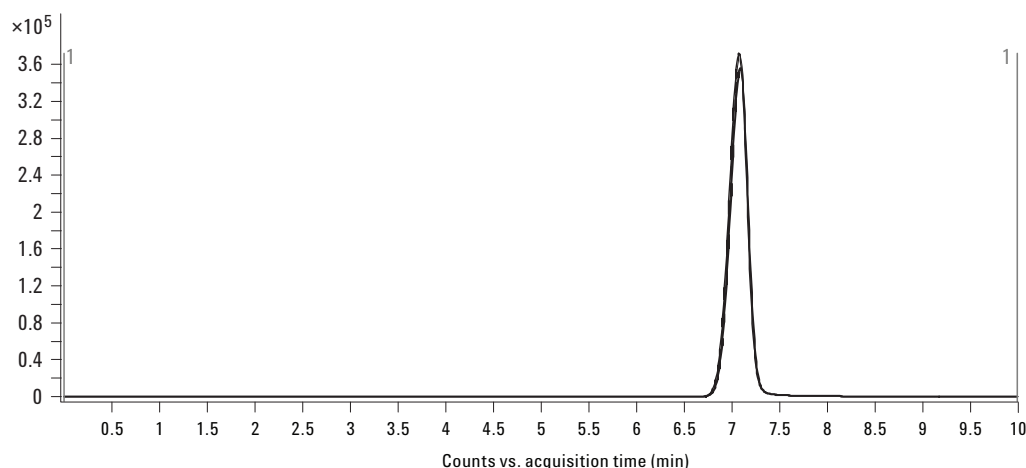


Figure 6. Replicate injections in liquid milk ($n = 3$) at 10 ppb level.

Table 1. Repeatability in Real Milk Samples with $n = 3$

Melamine concentration in real milk sample (ppm)	RSD of retention time (%)	RSD of MS response (%)
1	0.30	1.15
100	0.04	1.02

Excellent repeatability of this method is shown in Figure 6 and Table 1. This can ensure good results, even after day-to-day analysis of running samples.

Recovery

Using a calibration curve based on melamine standard in solvent, recovery data in milk powder is shown in Table 2.

Table 2. Recovery in Milk Powder

	Conc. = 80 ppb ($n = 3$)	Conc. = 50 ppb ($n = 3$)
Recovery (%)	62.5	83.4
RSD (%)	1.02	2.78

Saturation of the MS detector is observed at about 100 ppb. Using an internal standard method is recommended for future analysis with stable isotope labeled melamine.

Conclusions

A sensitive and specific method for the detection and quantitation of melamine in milk and milk products has been demonstrated. The method is robust and allows for the analysis of a large number of samples in complex matrices. Derivatization is not needed, and the method provides confirmation and quantitation in a single analysis at very low detection limits. This method can be readily used for confirmation of positive results obtained with less selective LC screening methods. The results of this study show that the Agilent 6410 LC/MS Triple Quadrupole, SampliQ SCX SPE cartridges, and a ZORBAX 300SCX HPLC column provide a robust, sensitive, and repeatable methodology.

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